Exhibit B

SIXTH EDITION

ESSENTIAL IMMUNOLOGY

Ivan M. Roitt

MA, DSc(Oxon), FRCPath, Hon MRCP (Lond), FRS

Professor and Head of

Departments of Immunology and Rheumatology Research

University College and Middlesex School of Medicine

University College

London W1P 9PG

BLACKWELL SCIENTIFIC PUBLICATIONS

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Blackwell Scientific Publications
Editorial offices:
Osney Mead, Oxford OX2 0EL
(*Orders*: Tel. 0865 240201)
8 John Street, London WC1N 2ES
23 Ainslie Place, Edinburgh EH3 6AJ
3 Cambridge Center, Suite 208, Cambridge
Massachusetts 02142, USA
107 Barry Street, Carlton, Victoria 3053, Australia

First published 1971
Reprinted 1972 (twice), 1973 (twice)
Second edition 1974, Reprinted 1975
Third edition 1977, Reprinted 1978, 1979
Fourth edition 1980, Reprinted, 1982, 1983
Fifth edition 1984
Sixth edition 1988, Reprinted 1988
Reprinted with corrections 1989

Spanish editions 1972, 1975, 1978, 1982
Italian editions 1973, 1975, 1979, 1986
Portuguese editions 1973, 1976
French editions 1975, 1979
Dutch editions 1975, 1978, 1982
Japanese editions 1976, 1978, 1982, 1986
German editions 1977, 1984, 1988
Polish edition 1977
Greek edition 1978
Slovak edition 1981
Indonesian edition 1985
Thai editions 1978, 1982, 1988

DISTRIBUTORS

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The C.V. Mosby Company
5240 Finch Avenue East,
Scarborough, Ontario
(*Orders*: Tel. 416-298-1588)

Australia
Blackwell Scientific Publications
(Australia) Pty Ltd
107 Barry Street
Carlton, Victoria 3053
(*Orders*: Tel. (03) 347 0300)

British Library
Cataloguing in Publication Data

Roitt, Ivan M.
Essential immunology. — 6th ed.
1. Immunology
I. Title
616.07'9 QR181

ISBN 0-632-01994-8

Set by Setrite Ltd, Hong Kong
Printed and bound by
Dah Hua Printing Press Co Ltd, Hong Kong

kine are capable of stimulating glucocorticoid synthesis and do so through the pituitary–adrenal axis.

IL-1 increases slow-wave sleep when introduced into the lateral ventricle of the brain and both IL-1 and interferon produce pyrogenic effects through their action on the temperature-controlling centre. The significance of IgFc binding to pituitary ACTH-producing cells has still not been revealed.

Circuits

Two network interactions between the immune and neuroendocrine systems have been reasonably well established. The first would involve the increased synthesis of glucocorticoids under the influence of IL-1, a lymphokine and possibly a thymus hormone, generated during the immunological response. In turn, the glucocorticoids would exert feedback suppression by influencing several processes including production of IL-1 and IL-2 (figure 8.15a).

A second circuit involves the seemingly intimate relationships between hormone receptor, hormone, anti-hormone and anti-idiotype (figure 8.15b). This point has come to our attention already in discussing the production of anti-receptor antibodies during immunization with an acetylcholine agonist (figure 8.7) and is of relevance to the pathogenesis of autoimmune disorders directed against hormone receptors (see p. 258).

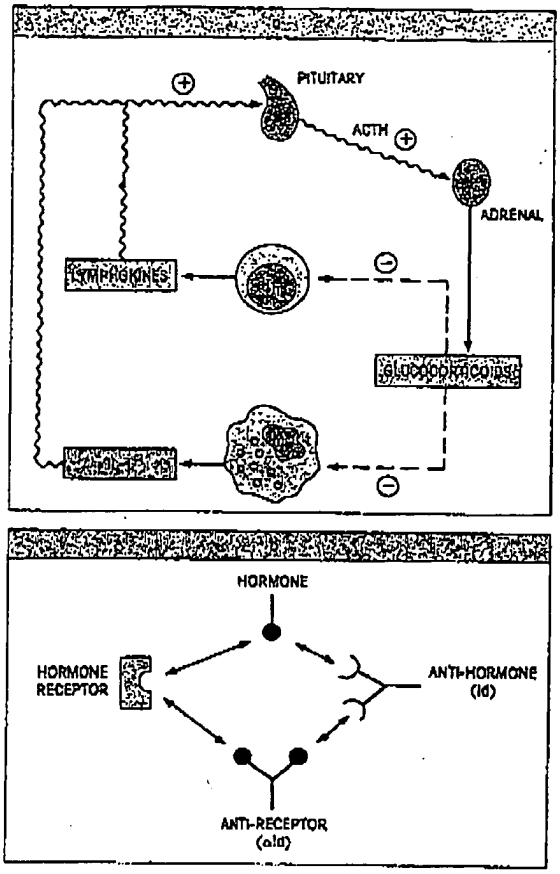

Figure 8.15. *Two immunological circuits involving neurological and endocrinological systems. (a) Glucocorticoid feedback through IL-1 and lymphokine production (after Besedovsky and colleagues). (b) Autoantibodies to hormones and their receptors (cf. figure 8.7).*

Events in the neonatal period

Over 40 years ago Owen made the intriguing observation that non-identical (dizygotic) twin cattle, which shared the same placental circulation and whose circulations were thereby linked, grew up with appreciable numbers of red cells from the other twin in their blood; if they had not shared the same circulation at birth, red cells from the twin injected in adult life would be rapidly eliminated by an immunological response. From this finding Burnet & Fenner conceived the notion that potential antigens which reach the lymphoid cells during their developing immunologically immature phase in the perinatal period can in some way specifically suppress any future response to that antigen when the animal reaches immunological maturity. This, they considered, would provide a means whereby unresponsiveness to the body's own constituents ('self') could be established and thereby enable the lymphoid cells to make the important distinction between 'self' and 'non-self'. On this basis, any foreign cells introduced into the body around the perinatal period should trick the animal into treating them as 'self' components in later life and the studies of Medawar and his colleagues have shown that *immunological tolerance* or unresponsiveness can be artificially induced in this way. Thus neonatal injection of CBA mouse cells into newborn A strain animals suppresses their ability to immunologically reject a CBA graft in adult life (figures 8.16

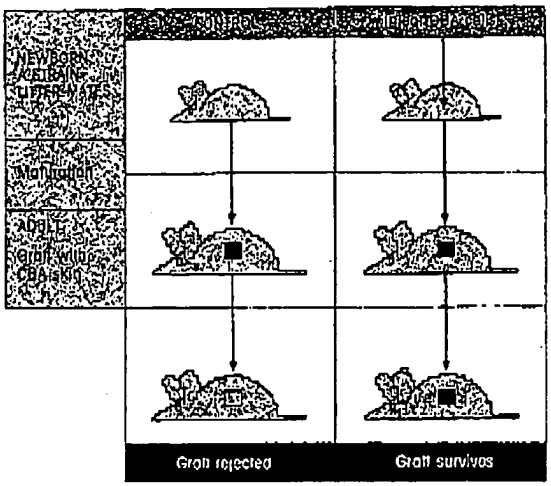

Figure 8.16. *Induction of tolerance to foreign CBA skin graft in A strain mice by neonatal injection of antigen (after Billingham R., Brent L. & Medawar P.B. (1953) Nature 172, 603). The effect is antigen specific since the tolerant mice can reject third-party grafts normally.*

and 8.17). Tolerance can also be induced with soluble antigens; for example, rabbits injected with bovine serum albumin at birth fail to make antibodies on later challenge with this protein.

Events in the adult

It is now recognized that tolerance can be induced in the adult as well as the neonate, although in general much higher doses of antigen are required (figure 8.18). Surprisingly, repeated injection of *low* doses of certain antigens such as bovine serum albumin (BSA) which are weakly immunogenic, established a state of tolerance as revealed by a poor antibody response on challenge with BSA in a strongly antigenic form (in complete Freund's adjuvant—see p. 181). It was shown subsequently that this 'low zone tolerance' could also be achieved with more powerful antigens provided an immunosuppressive drug such as cyclophosphamide was given to inhibit antibody synthesis during the low dose treatment.

Thus, there is a 'low zone' and a 'high zone' in terms of antigen predosage for tolerance induction. Elegant studies by Weigle and co-workers have pin-pointed the T-cell as the target for tolerance at low antigen levels while both B- and T-lymphocytes are made unresponsive at high antigen dose (table 8.4). Thus, for 'thymus-dependent' antigens at dose levels where the T-cells play a major cooperative role in antibody formation, the overall immunological performance of the animal will reflect the degree of reactivity of the T-cell population. In other words, the T-cells guide the reaction and when they are tolerant the B-cells will not respond. The implications for self-tolerance are that the concentrations at which autologous molecules circulate, will determine the cellular compartment which is tolerized (figure 8.19).

Protein antigens are more tolerogenic (able to induce tolerance) when in a soluble rather than an aggregated or particulate form which can be readily taken up by macrophages, and it seems that molecules are more likely to be tolerogenic if they escape processing by macrophages before presentation to

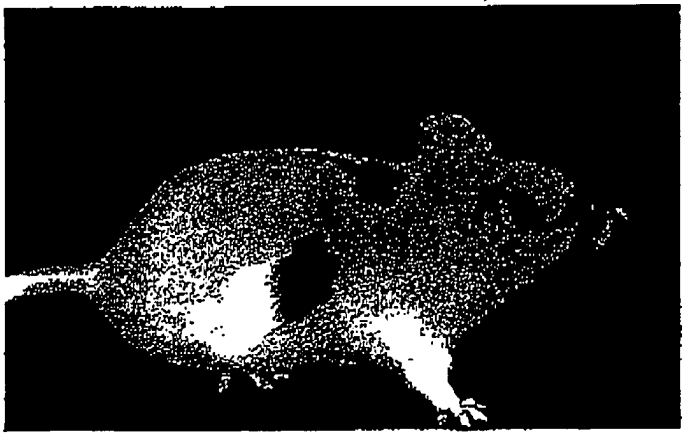

Figure 8.17. *CBA skin graft on fully tolerant A strain mouse showing healthy hair growth eight weeks after grafting (courtesy of Prof. L. Brent).*

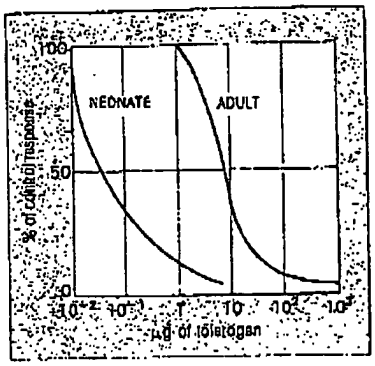

Figure 8.18. *Relative susceptibility of neonatal versus adult B-cells to tolerization by different doses of T-dependent antigen fluorescein-conjugated human IgG. The degree of tolerance was assessed by the antibody response relative to controls after challenge with antigen. Neonatal B-cells are tolerized at concentrations 100-fold lower than those effective against adult cells.*

the lymphocyte. Persistence of antigen is required to maintain tolerance. In Medawar's experiments the tolerant state was long lived because the injected CBA cells survived and the animals continued to be chimaeric (i.e. they possessed both A and CBA cells). With non-living antigens such as BSA, tolerance is gradually lost, the most likely explanation being that, in the absence of antigen, newly recruited immunocompetent cells which are being generated throughout life are not being rendered tolerant. Since recruitment of newly competent T-lymphocytes is drastically curtailed by removal of the thymus, it is of interest to note that the tolerant state persists for much longer in thymectomized animals.

Table 8.4. *Effect of antigen dose on tolerance induction in T- and B-cells.*

Tolerogen administered (mg)	% Tolerance induced		
	T-cells	B-cells	DONOR SPLEEN
0.1	96	9	62
0.5	99	56	97
2.5	99	70	99

After induction of tolerance to aggregate-free human IgG in mice, the reactivity of thymocytes and bone marrow cells (containing B-cells) was assessed by transfer to irradiated recipients of either bone marrow or thymus respectively from normal donors followed by IgG in an immunogenic form. The degree of tolerance induced in the donor is shown in the final column. Low antigen doses tolerize the T-cells. B-cells become unresponsive at higher doses. The T-cell activity largely dictates the response of the spleen as a whole (from Chiller J.M., Habicht G.S. & Weigle W.O. (1971) Science 171, 183).

Mechanism of tolerance induction

Immunological 'silence'

It is self-evident that the immune system will be tolerant of its own body components if it cannot communicate with them—we might then speak of a state of immunological 'silence'. Since virtually all body components are likely to be T-dependent because they lack the molecular qualities of T-independence (p. 94), they will not become immunogenic unless they associate with class II MHC. Soluble circulating materials can become associated with class II with some efficiency through capture and processing by B-cells; however, if there is a relatively low concentration of the component *and* the B-cells have a relatively low affinity, the signal to the T-helpers will probably not be adequate for triggering. Turning to surface molecules on cells which normally lack class II, e.g. TSH receptors on the thyroid, it is clear that 'silence' will dominate since they would be incapable of activating self-reactive T-cells (unless they were shed from the surface in large amounts and then presented by class II positive macrophages).

Immunological silence would also result if an individual has no genes coding for lymphocyte receptors directed against particular self-determinants; analysis of the experimentally induced autoantibody response to cytochrome *c* suggests that only those parts of the molecule which show species variation are autoantigenic whereas the highly conserved regions where the genes have not altered for a much longer time appear to be silent, supposedly because the autoreactive specificities have had time to disappear.

Clonal deletion

The restriction of each lymphocyte to a single specificity makes the job of establishing self-tolerance that much easier simply because it just requires a mechanism which functionally deletes self-reacting cells and leaves the remainder of the repertoire unscathed. The mechanism requires self-antigens to select the relevant clones since there is no other way to recognize them. The exceptional vulnerability of the neonate to tolerance induction has led to the suggestion that, during lymphyocyte development, the cell goes through a phase in which contact

with antigen leads to death or permanent inactivation. In support of this view, the surface Ig of very early B-cells can be capped at much lower concentrations of anti-IgM than are required by adult cells and remarkably, after endocytosis of the caps, the surface receptors are resynthesized by the adult cells but not by the early B-lymphocytes which are now effectively aborted through their inability to 'see' antigen. Even if they do ultimately resynthesize their receptors, they can still have been functionally inactivated; mice tolerized at birth with DNP linked to human IgG grow up with B-cells which can be isolated on DNP-immunoadsorbents through their surface receptors, yet cannot be activated to make anti-DNP as can similarly isolated cells from normal animals. Like the aged roué wistfully drinking in the visual attractions of some young belle, these tolerized lymphocytes can 'see' the antigen but lack the ability to do anything about it.

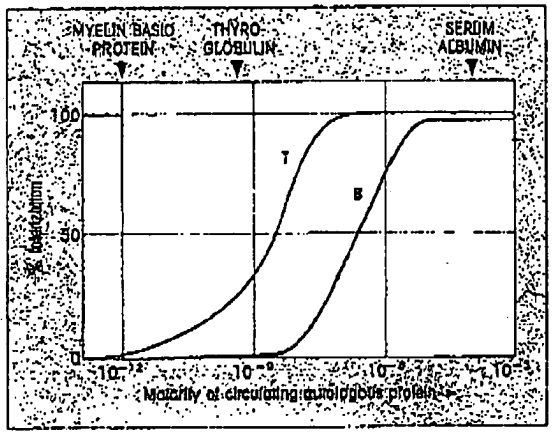

Figure 8.19. *Relative susceptibility of T- and B-cells to tolerance by circulating autologous molecules. Those circulating at low concentration induce no tolerance; at intermediate concentration, e.g. thyroglobulin, T-cells are moderately tolerized; molecules such as albumin which circulate at high concentrations tolerize both B- and T-cells.*

An ingenious mechanism based upon the idea of a 'veto' cell has been introduced to eliminate autoreactive cytotoxic T-cells, it being envisaged that such cells have the power to make cytolytic T-cells which recognize them commit suicide but leave the 'veto' cell unharmed. Concrete evidence that certain T-cell receptor V_β families are deleted by contact with self antigens in the thymus will be discussed in the following chapter.

Tolerance can be maintained by T-suppressor cells

Tolerance to protein antigens, induced at low concentrations, has been shown in at least one case to be mediated by T-suppressors directed against T-helpers and a suppressor mechanism will probably prove to be the most common basis for this phenomenon. The inferior immunogenicity of soluble, as distinct from aggregated or particulate, antigen has been ascribed to weak stimulation of T-helpers in contrast to effective activation of suppressor cells.

Helplessness of B-cells

We have already seen (cf. figure 8.19), that T-cells are more readily tolerized than B-cells and, as a result, a number of self-reacting B-cells are present in the body which cannot be triggered by T-dependent self-components since the T-cells required to provide the necessary T—B help are already tolerant —you might describe the B-cells as helpless. If we think of the determinant on a self-component which combines with the receptors on a self-reacting B-cell as a hapten and another determinant which has to be recognized by a T-cell as a carrier (cf. figure 6.10), then tolerance in the T-cell to the carrier will prevent the provision of T-cell help and the B-cell will be unresponsive.

If Bretscher and Cohn are correct in their hypothesis that two signals are needed for B-cell triggering but that a single signal through the Ig receptor alone leads to tolerance, there may be further consequences of helplessness, because if the self-reactive determinants cross-link B-cell receptors in the absence of T-cell signals, the B-cell should become tolerized; in fact, B-cell tolerance to haptenic determinants is readily produced when the hapten is presented to the B-cell on a thymus-independent carrier or on a carrier such as autologous IgG, to which the individual is already tolerant.

The concept of a single signal leading to tolerance may be extended to T-cells since there is evidence that if they are stimulated in the absence of IL-2 or in the presence of antibodies to MHC class II, prolonged unresponsiveness will be the outcome.

It is likely that self-tolerance involves all these mechanisms to varying degrees and that while clonal deletion is of prime importance early in life, T-suppression becomes a dominant factor later (figure 8.20). It should be stressed that these terms, early and late, apply to the life of the lymphocyte, not of the host. If an adult is irradiated and reconstituted with immature lymphocytes in the form of

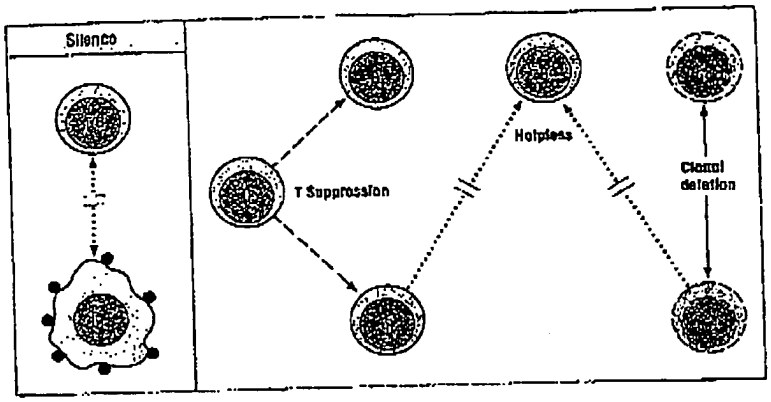

Figure 8.20. *Mechanisms of self-tolerance. Unresponsiveness will result if self-components and lymphocytes will not speak to each other (self-determinant (●) on Ia negative cell or lymphocyte lacks receptors), if T- or B-cells are T-suppressed or clonally deleted, or if T-dependent B-cells are deprived of T-help.*

bone marrow cells, the animal behaves as a neonate with respect to the ease of tolerance induction with low doses of antigen.

SUMMARY

Regulation of the antibody response is strongly influenced by antigen concentration; since the response is largely antigen driven, as effective antigen levels fall through catabolism and antibody feedback, the synthesis of antibody wanes.

T-cells regulate B-lymphocyte responses not only through cooperative help but also by T-cell suppressor networks which are initiated by inducer cells of T-helper phenotype. There is class II restriction governing these interactions but the precise molecular mechanisms are uncertain. Antigen-specific, idiotype-specific and non-specific suppressors all contribute. Effector T-cells are guided to their targets by MHC surface molecules, cytotoxic T-cells by class I, T-helpers by class II and T-suppressors by 'I-J'.

Lymphocytes can interact with the idiotypes on the receptors of other lymphocytes to form a network (Jerne). Idiotypes which occur frequently and are shared by a multiplicity of antibodies (public or cross-reacting Id) are targets for regulation by anti-idiotypes in the network thus providing a further mechanism for control of the immune response. The network offers the potential for therapeutic intervention to manipulate immunity.

A number of varied genetic factors influence the immune response. Approximately ten genes control the overall antibody response to complex antigens: some affect macrophage antigen handling and some the rate of proliferation of differentiating B-cells. Genes coding for antibodies of given specificities may be inherited together with (i.e. linked to) genetic markers for the heavy chain. Immune response genes linked to the major histocompatibility locus define the class II products on the T- and B-cells and antigen-presenting cells which control the interactions required for T–B collaboration.

Immunological, neurological and endocrinological systems can all interact and regulatory interdependent circuits are being described.

Immunological tolerance can be induced by exposure to antigens in neonatal and (less readily) in adult life. T-cells are more readily tolerized than B-cells leaving T-dependent B-cells 'helpless'. Elimination of specific cells or generation of T-suppressors may occur, while unresponsiveness to some self-components results from immunological 'silence'.

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